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Cdc14 Phosphatase Induces rDNA Condensation and Resolves Cohesin-Independent Cohesion during Budding Yeast Anaphase

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Summary

At anaphase onset, the protease separase triggers chromosome segregation by cleaving the chromosomal cohesin complex. Here, we show that cohesin destruction in metaphase is sufficient for segregation of much of the budding yeast genome, but not of the long arm of chromosome XII that contains the rDNA repeats. rDNA in metaphase, unlike most other sequences, remains in an undercondensed and topologically entangled state. Separase, concomitantly with cleaving cohesin, activates the phosphatase Cdc14. We find that Cdc14 exerts two effects on rDNA, both mediated by the condensin complex. Lengthwise condensation of rDNA shortens the chromosome XII arm sufficiently for segregation. This condensation depends on the aurora B kinase complex. Independently of condensation, Cdc14 induces condensin-dependent resolution of cohesin-independent rDNA linkage. Cdc14-dependent sister chromatid resolution at the rDNA could introduce a temporal order to chromosome segregation.

Introduction

Faithful chromosome segregation is fundamental to the inheritance of eukaryotic genomes during growth and proliferation. Chromosomes are replicated during S phase of the cell cycle, and exactly one copy must be segregated into each daughter cell during mitosis. Newly synthesized sister chromatids remain linked to each other, such that pairs of chromatids can be aligned in mitosis on the bipolar spindle for segregation in opposite directions. The nature of a postreplicative link, or cohesion, between sister chromatids has been considered for a long time and has become the subject of intense study over recent years (Nasmyth et al., 2000).

After termination of replication, sister strands remain topologically linked to each other by catenation, and this could in principle provide cohesion between sister pairs until segregation during anaphase (Murray and Szostak, 1985). The activity of topoisomerase II, responsible for DNA decatenation (henceforth called "topo II"), is indeed required during anaphase for complete sister chromatid segregation, but it does not appear to regulate the onset of anaphase (Downes et al., 1991; Holm et al., 1985; Shamu and Murray, 1992; Uemura et al., 1987). Furthermore, stable maintenance of minichromosomes does not depend on catenation in mitosis (Koshland and Hartwell, 1987), suggesting that other means to regulate chromosome segregation exist.

The discovery of a proteinaceous molecular "glue" that connects sister chromatids, the chromosomal cohesin complex, has provided an alternative explanation of sister chromatid cohesion and segregation (Guacci et al., 1997; Michaelis et al., 1997; Nasmyth et al., 2000). Cohesin binds to DNA before replication and ensures that replication products are linked after their synthesis. Delaying sister chromatid decatenation after DNA replication cannot defer the requirement for cohesin (Uhlmann and Nasmyth, 1998). Cohesin remains chromatin bound in metaphase, when it provides the counterforce for bipolar alignment of sister chromatid pairs on the mitotic spindle. Anaphase onset is triggered when separase, a site-specific protease, is activated and cleaves cohesin's Scc1 subunit (Uhlmann et al., 2000). Cohesin forms a large proteinaceous ring, and an intriguing possibility therefore is that it encircles and topologically traps sister chromatids (Anderson et al., 2002; Haering et al., 2002).

In the absence of cohesin, yeast chromosomes show premature separation and random segregation during mitosis (Guacci et al., 1997; Michaelis et al., 1997). Moreover, engineered Scc1 cleavage in metaphase-arrested budding yeast cells, using the foreign TEV protease, triggers the formation of binucleate cells and segregation of a GFP-marked *URA3* locus on chromosome V (Uhlmann et al., 2000). This demonstrates the importance of cohesin for sister chromatid cohesion and suggests that cohesin cleavage in anaphase is sufficient to split much, if not all of the genome.

An important requirement for successful segregation in mitosis is chromosome condensation, mediated by the chromosomal condensin complex (Bhat et al., 1996; Losada and Hirano, 2001; Saka et al., 1994). Even the relatively small budding yeast chromosomes, at their interphase level of compaction, would be too long to be efficiently segregated (Guacci et al., 1994). Mitotic condensation leads to an approximately 2-fold shortening of budding yeast chromosome arms, which ensures their complete movement across the bud neck. Furthermore, condensation is thought to facilitate the individualization of sister chromatid axes in preparation for segregation. In the absence of condensin, sister chromatids remain intertangled and fail to separate. This has been attributed to persisting catenation between sister chromatids which condensation may help to resolve, although no direct evidence for this has so far been obtained.

When staining for a nucleolar antigen after TEV protease-induced cohesin cleavage it came to our surprise that, in contrast to the bulk of DNA, the nucleolus did

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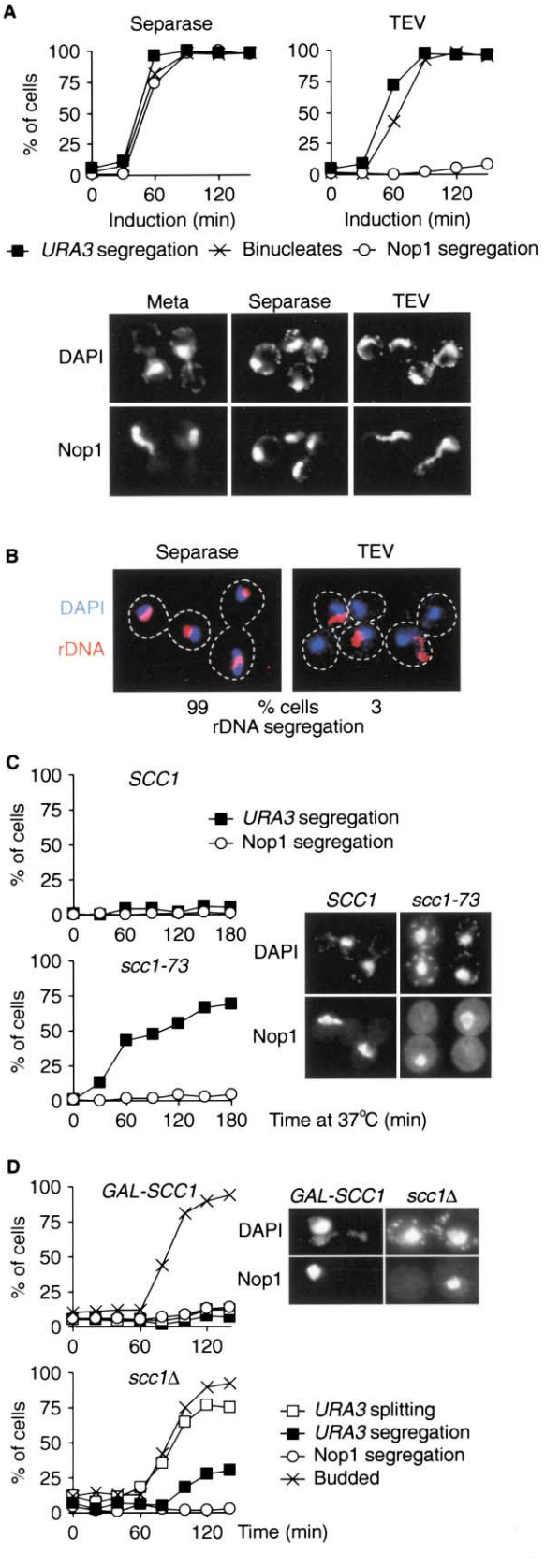

Figure 1. Cohesin Cleavage Is Not Sufficient for rDNA Segregation

(A) The nucleolus does not segregate after TEV protease-mediated cohesin cleavage. Strains Y116 (*MATalpha MET3-CDC20 scc1Δ SCC1^{TEV} GAL-TEV TetOs::URA3 TetR-GFP*) and Y121 (*MATa MET3-CDC20 GAL-ESP1 TetOs::URA3 TetR-GFP*) were arrested in metaphase (Meta) by depletion of Cdc20, and separase or TEV protease expression was induced. Chromosome segregation was analyzed by scoring segregation of the GFP-marked *URA3* locus and DAPI staining. The nucleolus was visualized by staining against Nop1 (Aris and Blobel, 1988). The photographs show cells 2 hr after induction.

(B) The rDNA does not segregate after TEV protease-mediated cohesin cleavage. rDNA FISH was performed on samples from (A), 2 hr after induction. The outline of cells, as seen on longer exposures, is indicated.

(C) The nucleolus does not segregate after temperature-sensitive cohesin inactivation. Strains Y1119 (*MATa GAL-CDC20 TetOs::URA3 TetR-GFP*) and Y1293 (as 1119 but *scc1-73*) were arrested in metaphase by Cdc20 depletion and shifted to 37°C to inactivate cohesin. Cells are shown 2.5 hr after the temperature shift.

(D) Nucleolar segregation fails after Scc1 depletion. Strains Y1342 (*MATa cdc26Δ TetOs::URA3 TetR-GFP*) and Y1341 (as Y1342 but *GAL-SCC1*) were arrested in G1 by α factor treatment and expression of Scc1 was repressed by shift to glucose-containing medium. Cells were released into the cell cycle at 37°C to arrest in metaphase due to the lack of Cdc26. Cells are shown 2 hr after release.

not efficiently segregate (Sullivan and Uhlmann, 2003). We now show that cohesin cleavage is not sufficient to allow segregation of the rDNA array on the long arm of chromosome XII, on which the nucleolus is assembled. We find that the rDNA in metaphase is less condensed than most other sequences and that this correlates with persisting catenation at this locus. The rDNA fully condenses and disentangles only during anaphase when the Cdc14 phosphatase is activated. Condensation and resolution of the rDNA during anaphase both depend on condensin, but are distinct as only the former depends on aurora B kinase.

Results

Cohesin Cleavage Is Not Sufficient for rDNA Segregation

We have analyzed the contribution of separase activation and cohesin cleavage to mitotic progression in budding yeast. Cells were arrested in metaphase by depletion of Cdc20, an essential activator of the anaphase-promoting complex (APC), and separase expression was induced from the galactose-inducible *GAL1* promoter. This triggered segregation of the *URA3* locus on chromosome V, and binucleate formation, as described (Figure 1A and Uhlmann et al., 2000). Staining against the nucleolar protein Nop1 (Aris and Blobel, 1988) confirmed that the nucleolus also split and segregated into daughters in all cells (Figure 1A). When instead of separase the foreign TEV protease was expressed to cleave cohesin, separation of the *URA3* locus and binucleate formation proceeded with similar efficiency. However, the Nop1-stained nucleolus did not segregate and became stretched across the bud neck (Figure 1A; Sullivan and Uhlmann, 2003).

The nucleolus is a complex macromolecular assembly that forms on the rDNA and is the site of a number of nuclear functions, including ribosome biogenesis (Sumner, 2003). The rDNA in budding yeast is an array of approximately 100 tandem repeats of a 9.1 kb rDNA unit on the long arm of chromosome XII. To investigate

whether the rDNA segregated after TEV protease induced cohesin cleavage, we performed fluorescence in situ hybridization (FISH) to visualize the locus. The rDNA segregated with daughter nuclei, stained with 4,6-diamidino-2-phenylindole (DAPI), in cells expressing separase (Figure 1B). It failed to segregate and remained in one mass between nuclei in cells expressing TEV protease. This suggests that cohesin cleavage is not sufficient to resolve sister chromatid cohesion at the rDNA. The rDNA stains poorly with DAPI, which explains why its segregation failure after TEV protease expression has not been noted previously.

The above result might be explained if TEV protease were unable to efficiently cleave cohesin in the nucleolus. However, cohesin staining on chromosome spreads and Western blotting did not provide any evidence for uncleaved cohesin remaining at the rDNA after TEV protease expression (data not shown). To confirm that cohesin destruction is not sufficient for nucleolar segregation, we inactivated cohesin in metaphase arrested cells carrying the temperature-sensitive *scc1-73* mutation (Michaelis et al., 1997). After temperature shift, two thirds of *scc1-73* cells showed bipartitioned nuclei and segregated *URA3* loci, but the nucleolus remained unsegregated (Figure 1C). We also depleted Scc1 under control of the repressed *GAL1* promoter in G1 arrested cells (Uhlmann and Nasmyth, 1998). These cells were then passed through a cell cycle in the complete absence of cohesin and arrested in metaphase by a temperature-sensitive APC mutation (Zachariae et al., 1996). Sister chromatid segregation is largely random after S phase without cohesin, and the *URA3* locus correctly segregated in one third of cells. In none of the cells did the nucleolus segregate (Figure 1D). This suggests that the rDNA is linked in metaphase by a form of cohesion that is independent of the cohesin complex.

Cohesin-Independent Cohesion at the rDNA Prevents Chromosome XII Segregation

We examined the consequences of rDNA cohesion for the segregation of chromosome XII. For this, we marked five loci along the chromosome with GFP (Straight et al., 1996): close to the left telomere on the short arm, the centromere, proximal and distal to the rDNA array on the long right chromosome arm, and toward the right telomere (see scheme in Figure 2). All loci separated and segregated into daughter cells when anaphase was triggered by separase expression in metaphase-arrested cells (Figure 2). When TEV protease was used to cleave cohesin, the two loci flanking the rDNA failed to separate, i.e., the GFP signal did not split in two. Loci at the centromere and left telomere, as well as on the right chromosome arm distal to the rDNA efficiently split. However, segregation of the separated loci into daughter cells was severely impaired (Figure 2). The centromere and left telomere of the chromosome entered daughter cells in a third of cells, but they never reached opposite ends of the cell. Separated sisters distal to the rDNA always stayed in close proximity within one of the cell halves. This suggests that rDNA sisters are held together independently of the cohesin complex, and that this linkage must be resolved for segregation of chromosome XII.

The rDNA is a heterochromatic locus that undergoes frequent recombination events, thought to be initiated at replication fork barriers in each repeat. To test whether any of these properties of the locus was the cause for cohesin-independent cohesion we deleted the genes encoding Sir2, an essential component of heterochromatin, Fob1 that maintains the replication fork barrier, and Rad52 required for homologous recombination. None of these alleviated cohesin-independent rDNA cohesion (data not shown). Further, the repetitive nature of the rDNA is not likely the sole reason for cohesion as repetitive telomeric sequences separate efficiently in response to TEV-protease induced cohesin cleavage (Figure 2 and Supplemental Figure S1 available at http://www.cell.com/cgi/content/full/117/4/471/DC1).

Cdc14 Activation Is Required and Together with Cohesin Cleavage Is Sufficient for rDNA Segregation

Separase, in addition to cohesin cleavage at anaphase onset, employs a protease-independent mode to activate the phosphatase Cdc14 (Sullivan and Uhlmann, 2003). Cdc14 is sequestered and inhibited in the nucleolus in metaphase from where it is released in anaphase (Shou et al., 1999; Visintin et al., 1999). Nucleolar segregation defects have been reported in *cdc14-1* mutant cells (Granot and Snyder, 1991), and we confirmed by FISH that indeed the rDNA failed to segregate in *cdc14-1* mutant cells arrested in telophase at the restrictive temperature (Figure 3A). The Cdc14-1 mutant protein is released from nucleoli similarly to wild-type Cdc14 (Jaspersen and Morgan, 2000) suggesting not the release, but the activity of released Cdc14 is important for nucleolar segregation.

We therefore asked whether Cdc14 activation together with cohesin cleavage was sufficient to allow rDNA segregation. We again expressed TEV-protease in metaphase-arrested cells to cleave cohesin, but now we coexpressed Cdc14. This permitted efficient segregation of rDNA to the daughter cells (Figures 3B and 3C). We excluded the possibility that ectopic expression of Cdc14 indirectly activated separase, as expression of Cdc14 alone did not initiate chromosome segregation or Scc1 cleavage (Figure 3B, and data not shown). The effect of Cdc14 depended on its phosphatase activity, as similar expression of a phosphatase-inactive Cdc14 protein (C283A) did not allow nucleolar segregation (Figures 3B and 3C). Cdc14 expression also allowed nucleolar segregation if cohesin was inactivated by the temperature-sensitive *scc1-73* mutation (Supplemental Figure S2 available on *Cell* website). This shows that Cdc14 activation together with cohesin cleavage is sufficient to resolve all cohesion at the rDNA.

Delayed Cdc14 Activation in *spo12Δ* and *slk19Δ* Mutants Uncouples Cohesin Destruction and Nucleolar Segregation

The above experiments suggest that two activities of separase, cohesin cleavage, and Cdc14 activation, together coordinate chromosome segregation. Activation of Cdc14 by separase is significantly delayed in cells lacking either of the proteins, Spo12 and Slk19 (Stegmeier et al., 2002). If nucleolar segregation in a normal cell cycle is indeed controlled by activation of Cdc14,

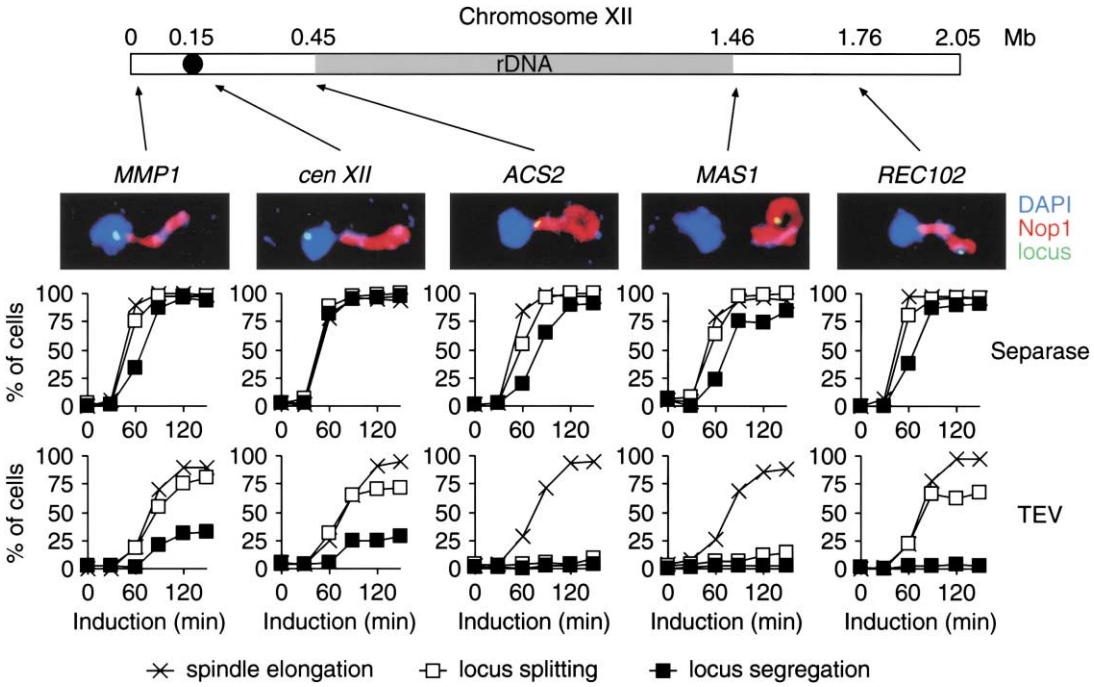

Figure 2. Chromosome XII Segregation during Anaphase Initiated by Separase or TEV Protease

Strains Y1780 (*MATa MET3-CDC20 GAL-ESP1 lacOs::MMP1 lacI-GFP*), Y1781 (*lacOs::cen XII*), Y1782 (*lacOs::ACS2*), Y1783 (*lacOs::MAS1*), and Y1784 (*lacOs::REC102*), as well as strains Y1785-Y1789 (*SCC1^{TEV} GAL-TEV*) respectively, were arrested in metaphase and separase or TEV protease was induced. A schematic map of the loci on chromosome XII is shown with an approximate rDNA length of 1 Mb. Representative images of each locus in metaphase are shown.

it should be delayed in cells lacking Spo12 or Slk19. To investigate this, we blocked wild-type, *spo12*Δ, and *slk19*Δ cells in metaphase by Cdc20 depletion and released them into synchronous anaphase by Cdc20 readdition. All three strains segregated the *URA3* locus and formed binucleate cells with similar kinetics. Wild-type cells showed nucleolar release of Cdc14 at the time of *URA3* separation, and the nucleolus separated about five minutes thereafter. Both Cdc14 release and nucleolar segregation were delayed by ten minutes in cells lacking Spo12 or Slk19 (Figure 4). Correlation of nucleolar segregation to anaphase spindle length in each cell revealed that nucleoli in *spo12*Δ and *slk19*Δ cells segregated when anaphase spindles had become significantly longer than in wild-type cells (Figure 4). This demonstrates that the timing of nucleolar segregation correlates closely with the time of Cdc14 activation. These results are consistent with the observation that separase, Spo12, and Slk19 are required for rDNA segregation in meiosis I (Buonomo et al., 2003).

Cdc14 Induces Condensation of the rDNA Array

We next investigated how Cdc14 affects the rDNA to promote segregation during anaphase. We noticed that expression of Cdc14 caused striking changes to the morphology of the rDNA. In metaphase cells, the rDNA visualized by FISH takes the shape of long "loop" structures (Guacci et al., 1994, 1997). We found the length of these loops to be 4.7 μm (s.d. 0.4 μm). Expression of Cdc14 caused compaction of these loops into significantly smaller "line"-like structures, 1.2 μm (s.d. 0.3 μm) in length (Figures 5A and 5B). This compaction depended on Cdc14's phosphatase activity (Figure 5B). Cdc14 is required for rDNA condensation also during normal anaphase, as rDNA appeared abnormally decondensed during anaphase in *cdc14-1* mutant cells (Figure 3A, and Guacci et al., 1994). In contrast, *cdc15-2* cells in anaphase presented line-like rDNA structures 2.1 μm (s.d. 0.2 μm) in length, indicating significant Cdc14-dependent rDNA compaction. Similar shortening of the rDNA during anaphase has recently also been seen in cells going through an undisturbed cell cycle (Lavoie et al., 2004).

We used Cdc14 expression in metaphase-arrested cells followed by FISH to examine which factors lie downstream of Cdc14 in inducing rDNA condensation. We tested the requirement of cohesin, condensin, topo II, and aurora B kinase, all of which have been implicated in chromosome condensation (Figure 5B). When metaphase-arrested *scc1-73* cells were shifted to 37°C, the rDNA lost its loop structure and instead formed decondensed "puffs,"' as reported (Guacci et al., 1997). In contrast, Cdc14-induced rDNA lines remained stable after shift to 37°C, indicating they are maintained independently of cohesin. This is consistent with the idea that Cdc14-dependent rDNA condensation normally occurs during anaphase after cohesin is destroyed.

The chromosomal condensin complex is essential for most if not all aspects of chromosome condensation (Takao et al., 2003), and a temperature-sensitive mutation in its Ycg1 subunit, *ycg1-10*, caused decondensation of metaphase loops, as expected (Figure 5B; Free-

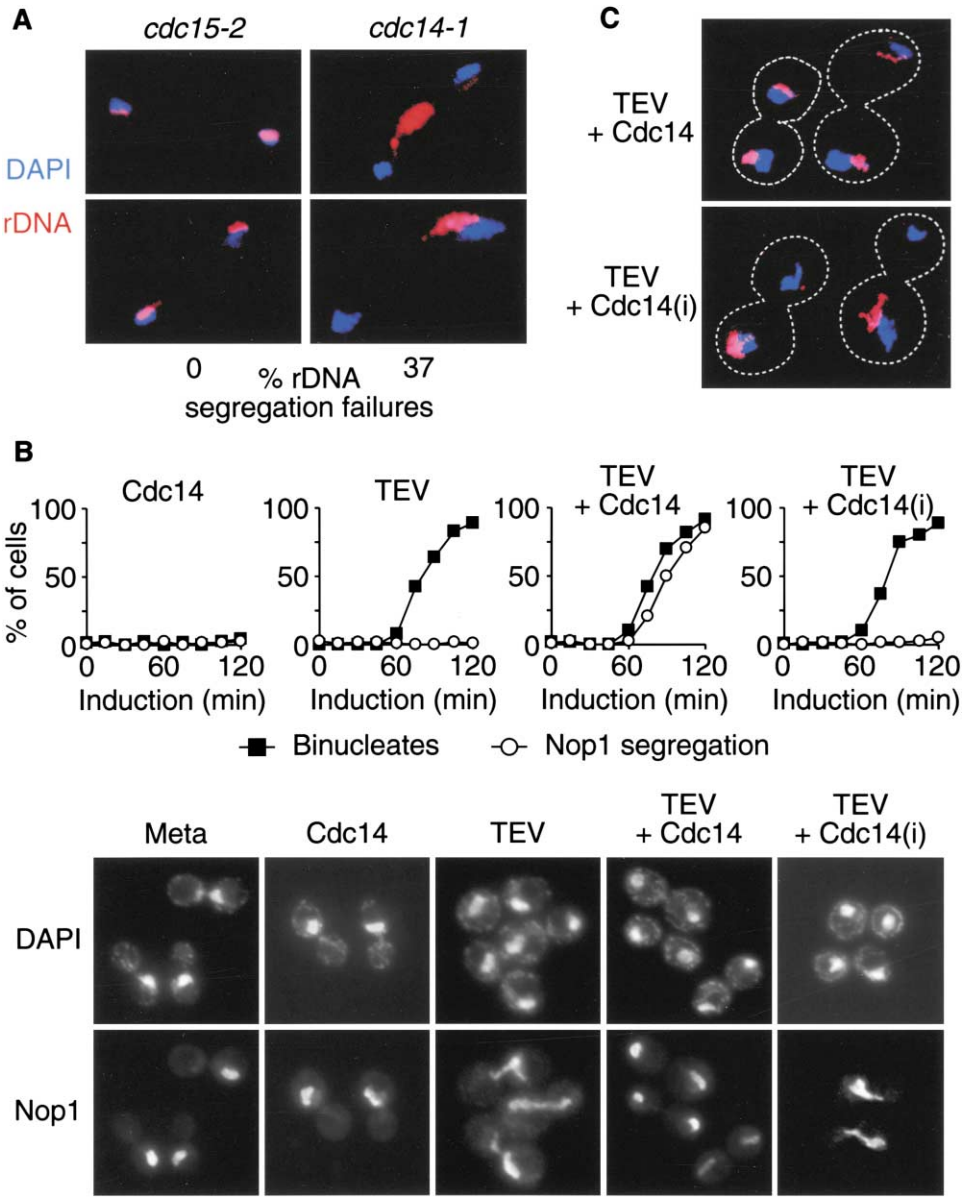

Figure 3. Cdc14 Phosphatase Is Required and with Cohesin Cleavage Is Sufficient for rDNA Segregation

(A) rDNA segregation fails in *cdc14-1* mutant cells. Strains Y1262 (*MATa cdc14-1*) and K1993 (*MATa cdc15-2*) were both arrested in telophase after 2.5 hr at 37°C, and rDNA FISH was performed.

(B) Cohesin cleavage and Cdc14 expression together allow nucleolar segregation. Strains Y353 (*MATalpha MET3-CDC20 scc1Δ SCC1^{TEV} GAL-TEV*), Y1239 (as 353 but *GAL-CDC14-Pk3*), Y1294 (as 353 but *GAL-CDC14(C283A)-Pk3*), and Y1304 (*MATalpha MET3-CDC20 GAL-CDC14-Pk3*) were arrested in metaphase (Meta) by Cdc20 depletion and expression of TEV protease and/or Cdc14 induced. Cells are shown 2 hr after induction. Similar expression of Cdc14 and phosphatase inactive Cdc14(i) was confirmed by Western blotting (data not shown).

(C) rDNA segregation after TEV protease-mediated cohesin cleavage and Cdc14 expression. FISH was performed on cells from (B) 2 hr after induction. rDNA segregated in 97% of cells expressing Cdc14, but in only 3% of cells expressing phosphatase inactive Cdc14.

man et al., 2000; Lavoie et al., 2000; Ouspenski et al., 2000). Cdc14-induced condensation also depended on condensin and was reduced to puffs in *ycg1-10* cells after shift to 37°C.

We found that topo II inactivation did not interfere with metaphase rDNA loops, as reported (Lavoie et al., 2002). rDNA lines after Cdc14 expression were also stable in *top2-4* cells, indicating that topo II activity is dispensable to maintain Cdc14-dependent condensation.

Aurora B kinase is required for budding yeast chromosome condensation after loss of cohesion at anaphase onset (Lavoie et al., 2004). We inactivated the budding yeast aurora B kinase complex using the *ipl1-321*, *ipl1-2*, or *sli15-3* temperature-sensitive alleles (Biggins et al., 1999; Kim et al., 1999), none of which had an effect on metaphase rDNA loops. However, further condensation after Cdc14 expression was abolished in all mutants and the rDNA remained in extended loops (Figure 5B, and data not shown). This suggests that Cdc14-induced rDNA condensation in anaphase requires aurora B ki-

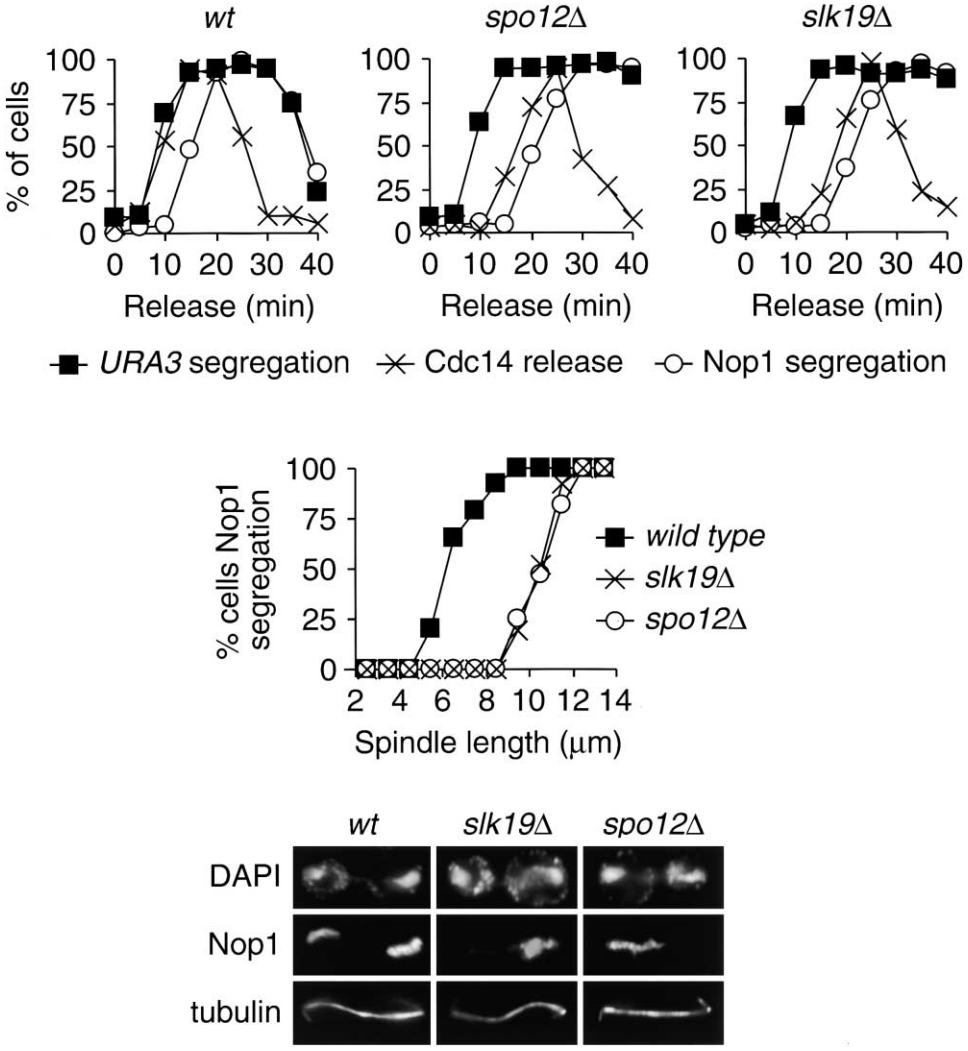

Figure 4. Cells Lacking Spo12 or Slk19 Show Delayed rDNA Segregation Corresponding to Delayed Cdc14 Activation

Strains Y1692 (*MATa GAL-CDC20 CDC14-HA6 TetOs::URA3 TetR-GFP*), Y1693 (as Y1692 but *slk19Δ*), and Y1694 (as Y1692 but *spo12Δ*) were arrested in metaphase by Cdc20 depletion and released into synchronous anaphase. Cdc14 nucleolar release and nucleolus segregation was monitored by indirect immunofluorescence. Lengths of randomly selected, stained anaphase spindles were measured using a graded ocular, and segregation of the Nop1 stained nucleolus was assessed. Cells shown are from 15 min after release.

nase. Cdc14 dephosphorylates the Sli15 subunit of aurora B kinase at anaphase onset, but mutation of six putative Cdk phosphorylation sites in Sli15 to alanine that abolish most mitotic phosphorylation (Pereira and Schiebel, 2003), was not sufficient to induce rDNA condensation (data not shown).

Condensin Function during Anaphase Is Required for rDNA Segregation

We next addressed the contribution of Cdc14-induced rDNA condensation to its segregation in anaphase. Mutations in budding yeast condensin cause severe chromosome segregation defects (Bhalla et al., 2002; Lavoie et al., 2000; Ouspenski et al., 2000). Condensin binds to rDNA and a specific role in transmission of this locus has been observed (Freeman et al., 2000). To address a possible role of Cdc14-induced rDNA condensation in anaphase, we inactivated condensin specifically during this time. We used two strains carrying temperature-sensitive mutations in condensin subunits (*smc2-8* [Freeman et al., 2000], and *ycg1-10*). These cells were arrested in metaphase at the permissive temperature to allow mitotic chromosome condensation to take place. After shifting to the restrictive temperature, cells were released into synchronous anaphase. Elongation of the anaphase spindle and formation of binucleate cells indicated that the role of condensin in segregation of most of the genome has been fulfilled by metaphase (Figure 6A). In striking contrast, the nucleolus did not segregate. Therefore, condensin is required during anaphase specifically for segregation of the rDNA. A Cdc14-induced, condensin-dependent reaction at the rDNA may therefore drive the resolution of cohesin-independent cohesion.

Topo II Is Essential for rDNA Resolution

Chromosome condensation is thought to aid the decatenation of intertangled sister chromatids. It has been

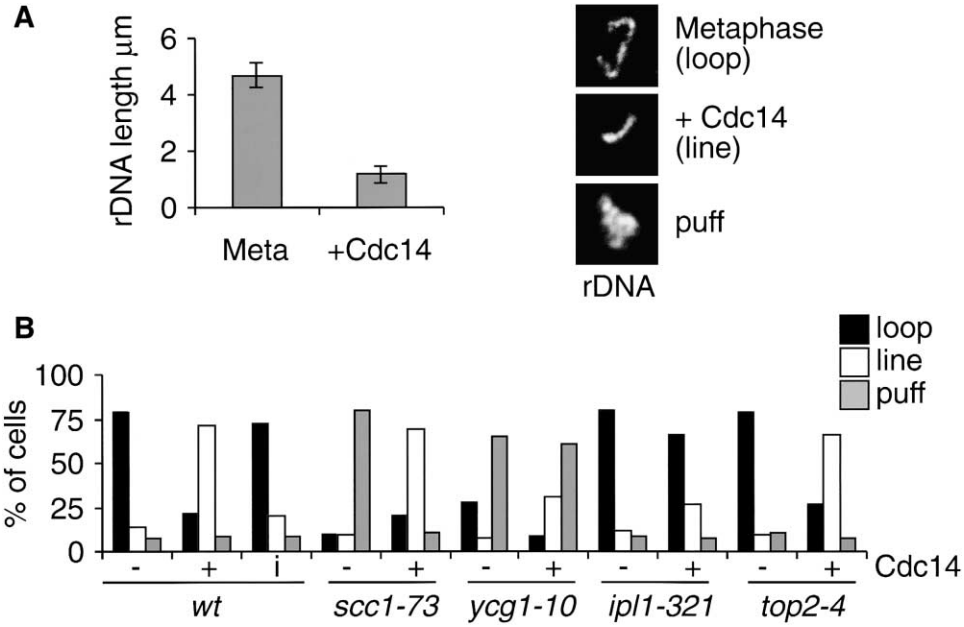

Figure 5. Cdc14 Induces rDNA Condensation Dependent on Condensin and Aurora B Kinase Function

(A) Cdc14 induces increased condensation of the rDNA array. Strains K699 (*MATa*) and Y1695 (*MATa GAL-CDC14-Pk3*) were arrested in metaphase by nocodazole treatment. Cdc14 was induced for 2 hr, and samples were prepared for rDNA FISH. The length of the rDNA locus was measured in 20 nuclei each, and the mean is presented with standard deviation. Examples of the metaphase loops and the shorter line-like structures after Cdc14 expression are shown, as well as a decondensed rDNA puff referred to in (B).

(B) Cdc14 induced rDNA condensation depends on condensin and aurora B kinase, but not cohesin or topo II. Strains Y1695, Y1697 (as Y1695 but *ycg1-10*), Y1698 (*top2-4*), Y1699 (*ipl1-321*), Y1700 (*scc1-73*), and Y1696 (*MATa GAL-CDC14(C283A)-Pk3*) were arrested in metaphase by nocodazole treatment and expression of Cdc14 (+) or phosphatase inactive Cdc14 (i) induced. Controls were left uninduced (−). 1 hr later cells were shifted to 37°C for 1 hr, then samples were prepared for rDNA FISH as in (A). Similar expression levels of Cdc14 and Cdc14(i) in all strains were confirmed by Western blotting (data not shown).

suggested condensin may recruit topo II to chromosomes, stimulate its activity, or simply provide directionality to the decatenation reaction by compacting individual sister chromatid axes (Bhalla et al., 2002; Bhat et al., 1996; Coelho et al., 2003). These scenarios remained hypothetical mainly for the fact that it is not actually known whether sister chromatid catenation is to blame for segregation failures observed due to condensation defects. Our finding that the rDNA fully condenses only during anaphase opened the opportunity to test whether condensation at this locus correlates with decatenation.

We used two independent alleles in the yeast topo II gene, *top2-4* and *top2-5*, both of which render decatenation activity of the enzyme temperature-sensitive (Jannatipour et al., 1993). Cells were grown and arrested in mitosis at the permissive temperature allowing topo II to be active during mitotic chromosome condensation. The temperature was then raised to inactivate topo II, and cells were released into synchronous anaphase. Nuclear division and segregation of the *URA3* locus proceeded apparently undisturbed, indicating that topo II-mediated decatenation was largely complete for much of the genome (Figure 6A). In contrast, the nucleolus failed to segregate and remained tightly linked between the divided nuclei. Thus, the requirement for topo II, and therefore most likely sister chromatid decatenation, correlates with condensation of the rDNA. This suggests that cohesin-independent cohesion at the rDNA is due to catenation that is resolved in a Cdc14-induced condensin-dependent step. Condensin and topo II are not required for Cdc14 activation at anaphase onset, excluding one alternative interpretation of our results (Supplemental Figure S3 available on *Cell* website).

rDNA Resolution Is Independent of Aurora B-Dependent Condensation

Aurora B kinase activity is required for rDNA condensation during anaphase (Figure 5B and Lavoie et al., 2004). If condensation was a prerequisite for decatenation then inactivation of aurora B should prevent rDNA segregation. However, rDNA segregation defects have not been reported in aurora B mutant strains. We found that *ipl1-321* mutant cells, in which aurora B kinase activity is undetectable at the restrictive temperature (Buvelot et al., 2003), were largely proficient in rDNA segregation despite defective condensation (Supplemental Figure S4 available on *Cell* website and Figure 6B). This suggests that Cdc14-induced condensin action on rDNA comprises two aspects. The first is condensin and aurora B-dependent rDNA condensation, leading to overall compaction of the locus. The second is condensin and topo II-dependent rDNA resolution, which is independent of aurora B kinase and the overall condensation.

Complete Chromosome XII Segregation Depends on Aurora B-Dependent rDNA Condensation

We addressed whether aurora B-dependent rDNA condensation, even though dispensable for rDNA resolution, played a role in chromosome XII segregation. We noticed that the length of rDNA loops in metaphase

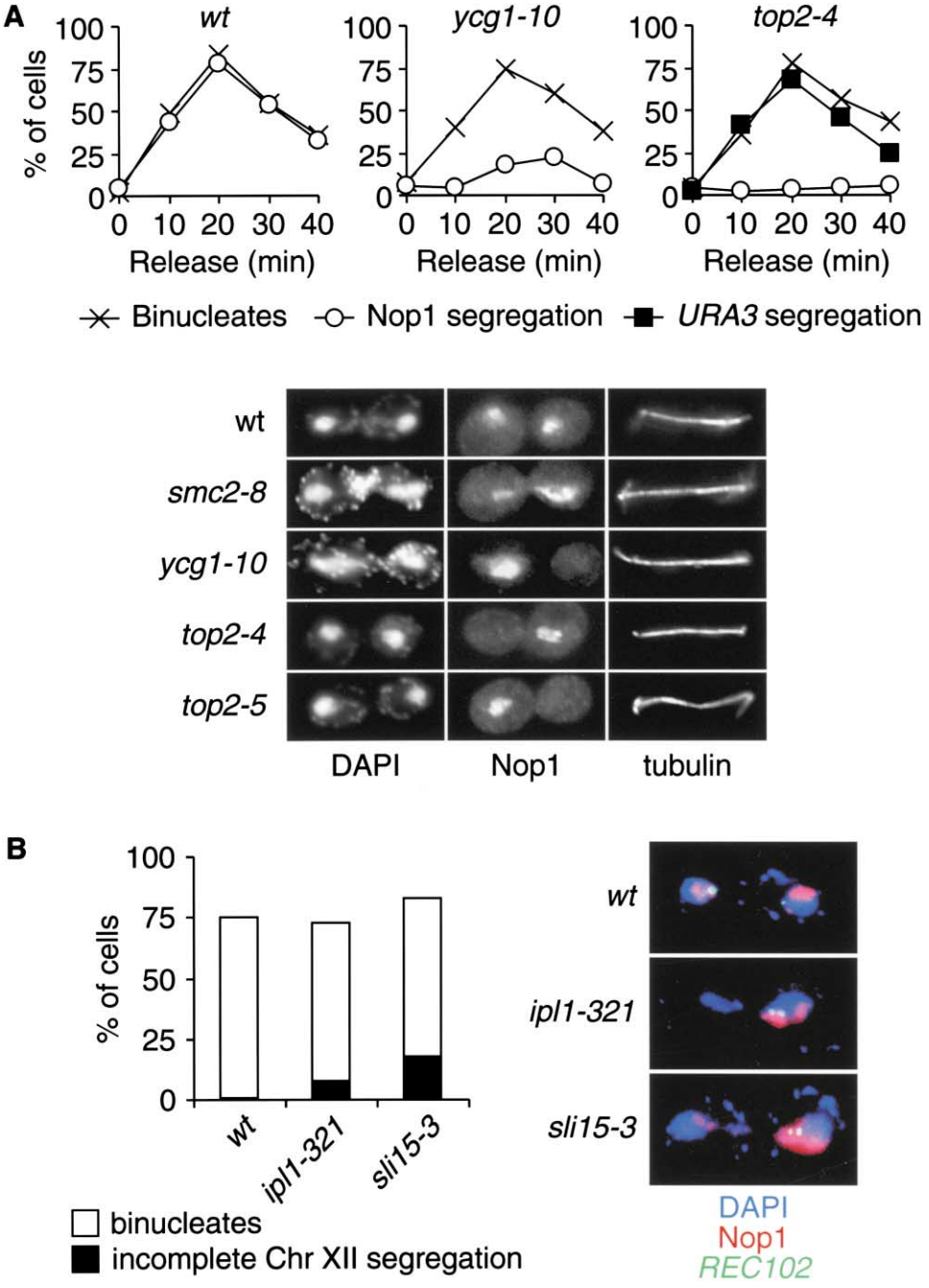

Figure 6. Two Condensin-Dependent Reactions Required for rDNA Resolution and Segregation

(A) Condensin and topo II are required during anaphase for resolution of the rDNA. Strains Y1119 (*MATa GAL-CDC20 TetOs::URA3 TetR-GFP*) and Y1702 (as Y1119 but *top2-4*), Y1334 (*MATa top2-5 GAL-CDC20*), Y1355 (*MATa smc2-8 GAL-CDC20*), and Y1336 (*MATalpha ycg1-10 GAL-CDC20*) were arrested in metaphase by Cdc20 depletion. Cells were shifted to 37°C for 1 hr before release into synchronous anaphase. Cells are shown 20 min after release.

(B) Aurora B-dependent condensation is not required for rDNA resolution but for efficient segregation of chromosome XII. Strains Y1784, Y1804 (as Y1784 but *ipl1-321)* and Y1805 (as Y1784 but *sli15-3*) were arrested in metaphase by Cdc20 depletion. Cells were shifted to 37°C to inactivate aurora B kinase and separase expression was induced. Chromosome XII segregation was analyzed 90 min after separase induction. Examples of *ipl1-321* and *sli15-3* cells that failed to segregate the *REC102* locus are shown.

(close to 5 μm) is half of the overall length of a budding yeast cell in mitosis (8-10 μm). The long arm of chromosome XII contains 900 kb of DNA in addition to the rDNA (Figure 2), and without significant shortening segregation of this arm would fail. We analyzed whether we could find evidence for incomplete chromosome XII segregation in cells lacking aurora B-dependent rDNA condensation. Because aurora B is required for the establishment of bipolar spindle attachment to sister kinetochores, we first arrested *ipl1-321* or *sli15-3* cells

in metaphase at the permissive temperature. Aurora B was then inactivated by temperature shift and separase expression induced. As expected, the nucleolus was able to partition in this experiment because aurora B-dependent condensation is not required for rDNA resolution. The GFP-marked *REC102* locus distal to the rDNA split, but failed to segregate into opposite cell halves in 11% of binucleate *ipl1-321* and 22% of binucleate *sli15-3* cells. Incomplete segregation of the *REC102* locus was seen in only 1% of wild-type cells. This indicates that aurora B-dependent rDNA condensation is important for shortening the long arm of chromosome XII sufficiently to allow its efficient segregation during anaphase.

Discussion

Cohesin-Independent Cohesion Persists at the rDNA

The chromosomal cohesin complex is a crucial mediator of sister chromatid cohesion. Cleavage of the cohesin subunit Scc1 by separase is thought to liberate sister chromatids at anaphase onset for bipolar segregation by the mitotic spindle. We now report the surprising finding that cohesin cleavage in budding yeast metaphase is sufficient for segregation of much of the genome, but not of the rDNA array on chromosome XII. Separase, at the same time as cleaving cohesin, activates the Cdc14 phosphatase, and Cdc14 activity is required and together with cohesin cleavage is sufficient for rDNA segregation (Figure 7). Similar findings have been made by Amon and coworkers (D'Amours et al., 2004).

The requirement for a Cdc14-mediated resolution step does not exclude that the cohesin complex also plays a role in rDNA cohesion. A cohesin association site is found in every rDNA repeat (Laloraya et al., 2000), and expression of Cdc14 did not cause visible rDNA splitting if cohesin was still intact in metaphase. This suggests that both the cohesin complex, as well as a cohesin-independent linkage, provide sister chromatid cohesion at the rDNA, and that both need to be removed for rDNA segregation in anaphase.

What is the nature of cohesin-independent rDNA cohesion? We found that segregation of the rDNA, in contrast to most other parts of the genome, strictly depended on topo II activity during anaphase. Topo II-mediated sister chromatid decatenation is an essential aspect of all chromosome segregation, but we now suggest that different parts of the genome have distinct decatenation timing during mitosis. The possibility that the rDNA remains catenated in metaphase could explain cohesin-independent linkage of this locus. Alternatively, cohesion might be mediated by a mechanism different from catenation, but resolution of that linkage could involve a step that requires topo II.

Previous studies found that inactivation of topo II during budding yeast anaphase leads to frequent chromosome nondisjunction, but not to a large increase in chromosome breaks that would be expected after forceful segregation of sporadically catenated sister strands (Holm et al., 1989). This is consistent with the existence of individual loci stably linked by persisting catenation.

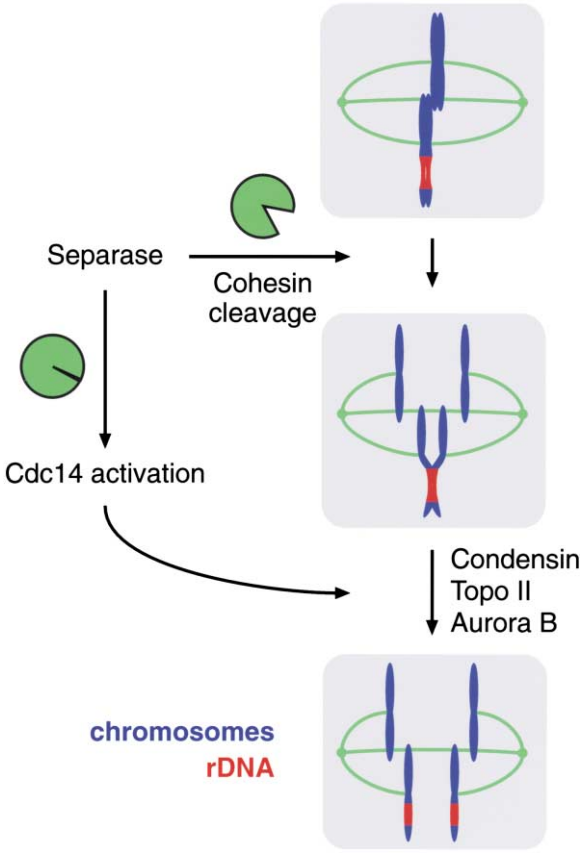

Figure 7. A Two-Step Model for Chromosome Segregation

Separase is activated at anaphase onset both as a protease to cleave cohesin, as well as in a protease-independent mode to activate the phosphatase Cdc14. Cohesin cleavage separates most parts of the genome (blue), but not the rDNA (red). Cdc14 activation induces rDNA condensation required for rDNA resolution and segregation. The delay that Cdc14 activation introduces to rDNA resolution may contribute to its late segregation.

Inactivation of fission yeast topo II at the metaphase to anaphase transition prevented completion of nuclear segregation by strong chromosome bridges (Uemura et al., 1987). Whether rDNA formed these bridges is not known. Studies to inactivate topo II specifically at anaphase onset in other organisms have been more difficult to perform (Downes et al., 1991).

rDNA Reaches Mitotic Condensation Only during Anaphase

The rDNA in metaphase, visualized by FISH, is organized in a loop-like structure, as compared to an apparently amorphous puff during interphase. This organization depends on condensin and is thought to reflect mitotic condensation of the locus (Lavoie et al., 2002). However, when we measured the length of the rDNA loop, we found it to be surprisingly long. At close to 5 μm, the loop is in fact too long to allow complete segregation of chromosome XII in an 8–10 μm long budding yeast cell. The compaction ratio of the approximately 1 Mb rDNA locus in metaphase calculates to 200 kb/μm, considerably lower than the 400-500 kb/μm found at eu-

chromatic loci in nocodazole-arrested cells (Guacci et al., 1994). Further lengthwise condensation of the rDNA during anaphase is therefore essential. We found that the rDNA shortens at least 2-fold during anaphase, i.e. it approaches the mitotic condensation of euchromatin. Chromosome condensation is thought to be induced by the high mitotic Cdk activity. Why full mitotic condensation of the rDNA depends on Cdc14 phosphatase, an opponent of Cdk activity, remains an intriguing puzzle.

Expression of Cdc14 in metaphase cells was sufficient to trigger rDNA condensation, which depended on both condensin and the aurora B kinase complex. The kinase activity of the aurora B complex is upregulated at the metaphase to anaphase transition in budding yeast (Buvelot et al., 2003), and it remains to be determined whether this is due to Cdc14. Cdc14 causes dephosphorylation of the Sli15 subunit in the aurora B kinase complex, but this may affect the localization rather than the overall activity of the kinase (Pereira and Schiebel, 2003). In search for other potential Cdc14 targets, we found that the Ycg1 subunit of condensin is modified by phosphorylation (data not shown, and Lavoie et al., 2004), however, this modification did not apparently change at anaphase onset. Amon and coworkers have found that condensin's Ycs4 subunit is sumoylated in a Cdc14-dependent fashion during mitosis (D'Amours et al., 2004).

Aurora B kinase is essential for the visible condensation, but not for condensin-dependent rDNA resolution in anaphase. This suggests that condensin has two roles, one in overall chromosome condensation that can be separated from another role that resolves catenated sister chromatids. This puts into question the idea that sister chromatid resolution is the consequence simply of compacting individual chromosome arms. Instead we find that, at least at the rDNA, condensin promotes the resolution of sister chromatids independently of overall condensation. It is possible that condensin promotes DNA reconfiguration at a level not visible by observation of the overall locus. Alternatively, condensin may recruit topo II or directly influence its activity at the rDNA. Results in support of a direct interaction between condensin and topo II have been obtained in yeast and *Drosophila*, but could not so far be confirmed in other organisms (Bhalla et al., 2002; Bhat et al., 1996; Coelho et al., 2003; Wignall et al., 2003).

Cdc14-Dependent rDNA Resolution Imposes a Temporal Pattern on Chromosome Segregation

Our studies raise the question why cohesin-independent linkage is maintained at the rDNA in metaphase. One possibility is that it is a mere consequence of incomplete rDNA condensation, although we do not know which aspect of nucleolar biology may benefit from reduced condensation in metaphase. In higher eukaryotes, mitotic condensation correlates with a reduction in transcriptional activity, but this is not the case in budding yeast. Specifically, rRNA transcription continues throughout mitosis (Elliott and McLaughlin, 1979), and we found that Cdc14-induced condensation did not reduce the levels of nascent rRNA transcripts (data not shown). We cannot exclude that another feature of the rDNA precludes its full condensation during metaphase.

Alternatively, cohesin-independent rDNA linkage may represent a bona fide form of sister chromatid cohesion. Catenation-based cohesion has been previously envisaged (Murray and Szostak, 1985), but carries the inherent risk of chromatid breakages if resolution is not perfectly efficient. This may be acceptable at a repetitive locus, such as the rDNA, where breaks would result in the loss only of redundant information. The introduction of a Cdc14 phosphatase-dependent step for resolution of rDNA cohesion could introduce a temporal pattern to the segregation of a complex genome (Figure 7). While cohesin cleavage is a direct consequence of separase activation, the pathway leading to Cdc14 activation and Cdc14-dependent resolution of cohesion introduces a delay. The rDNA is invariably last to segregate during budding yeast anaphase, and this might define the position of daughter nucleoli always in the nuclear periphery. A temporal pattern of segregation has also been observed in mammalian genomes (Gerlich et al., 2003; Vig, 1981), and whether the resolution of cohesin-independent cohesion is involved will be interesting to address.

Experimental Procedures

Yeast Strains and Plasmids

All strains were derivatives of W303 except Y1355 and Y1334, containing the *smc2-8* and *top2-5* alleles that were in the S288c background. The *ycg1-10* allele was isolated in a screen for EMS-induced temperature-sensitive mutations that show synthetic lethality with Scc1 in which one of the two separase cleavage sites was uncleavable due to the R268D mutation. This screen for "*spu*" (synthetic lethal with partially uncleavable Scc1) mutations recovered alleles in a number of proteins involved in DNA metabolism and chromosome segregation. The *spu4-1* mutation was found to be allelic with *YCG1*, and therefore renamed *ycg1-10*. Epitope tagging of endogenous genes and gene deletions were performed by gene targeting using polymerase chain reaction (PCR) products (Knop et al., 1999; Wach et al., 1994). Plasmids for integration of lacO arrays were created by cloning PCR fragments corresponding to loci on chromosome XII into plasmid pAFS163 (a kind gift from A. Straight). Fragments spanned the following chromosomal positions (as annotated in the *Saccharomyces Genome Database*) *MMP1*: 19776-21079, *YLR003c-1* (next to *cen XII*): 156860-157816, *ACS2*: 444533-445502, *MAS1*: 490606-491746, and *REC102* 786169-787284. The plasmids were integrated after linearization at unique restriction sites within the inserts (Kpn1, Kpn1, Sma1, Mfe1, and BsrG1, respectively). Wild-type or C283A mutant Cdc14 was cloned under the control of the *GAL1* promoter into YIplac vectors (Gietz and Sugino, 1988) using PCR, including a Pk epitope tag for detection at the C terminus.

Experimental Details

The expression of separase or TEV protease to trigger anaphase in cells arrested in metaphase by Cdc20 depletion under control of the *MET3* promoter was described previously (Uhlmann et al., 2000), as were the conditions for growth and release of synchronous cultures from arrest in G1 by α-factor (Sullivan et al., 2001). Arrest in metaphase by Cdc20 depletion under control of the *GAL1* promoter and release into anaphase by Cdc20 reinduction was as described (Uhlmann et al., 1999). At each time point of all experiments, 100 or more cells were analyzed to calculate percentages as presented. rDNA FISH was performed essentially following published procedures (Guacci et al., 1994; Guacci et al., 1997). The length of the rDNA array was measured on photographs of FISH and in situ stained samples and standardized against an image of a stage graticule. The antibodies used were α-Nop1 clone A66 (a kind gift from J. Aris, Aris and Blobel, 1988), α-tubulin clone YOL1/34 (Serotec, Oxford, UK), α-GFP clone TP401 (Torrey Pines Biolabs, Houston, TX), α-HA clone 12CA5, and α-Pk clone SV5-Pk1 (Serotec).

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